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Influence of Soil Acidity on the Occurrence of Athiorhodaceae

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ABSTRACT — Enrichment cultures from strongly acid *Sphagnum* bog peats and moderately acid to circumneutral fen peats revealed Athiorhodaceae to be present in almost all suitably wet samples above pH 5.3 and absent from almost all samples below pH 4.7. These bacteria were recorded in only one of fifty cultures from acid humus layers in a jack pine forest, and in none of thirty cultures from moderately acid humus layers in a mixed woodland. They were also absent from old-field surface soils, which often contained considerable amounts of organic litter.

A previous paper (Pratt and Gorham, 1970) recorded the prevalence of non-sulfur, purple photosynthetic bacteria (Athiorhodaceae) in generally organic surface soils along a soil moisture gradient from upland old fields and woods through cedar and tamarack swamps to a shallow pond. As expected, the bacteria were most prevalent in the wet sites, but they also were found occasionally in dry sites. This paper continues the earlier study by comparing the occurrence of Athiorhodaceae in circumneutral and acid environments.

The definitions of wetland environments used here are those of the Uppsala school (DuRietz 1949, Sjörs 1963), with bog representing *ombrotrophic* surfaces receiving mineral nutrients only from atmospheric precipitation, and fen representing *minerotrophic* surfaces receiving mineral nutrients from waters which have percolated through mineral soil.

Surface samples of organic matter were collected (with disposable Parke-Davis examination gloves well washed in alcohol) on 6 November 1969 and 4 June 1970 from strongly to moderately acid soils at a site seven miles north of Marcell, in northern Minnesota. A transect was followed from jack pine (*Pinus banksiana*) forest on an acid podzol soil to a bog with scattered black spruce (*Picea mariana*) and tamarack (*Larix laricina*) on acid *Sphagnum* peat. In the *Sphagnum* sites living moss was included in the peat samples. At the margin of the bog is a shrubby fen with willow, alder, sedges and grasses (= *lagg* in Swedish terminology).

The pinewood ground flora includes as characteristic species *Pteridium aquilinum, Gaultheria procumbens, Arctostaphylos uva-ursi, Maianthemum canadense, Chimaphila umbellata, Lycopodium complanatum, Calliergonella schreberi,* and large patches of *Cladonia* spp. The bog flora includes *Sphagnum magellanicum* and especially *S. fuscum* as hummock-forming species, with *Ledum groenlandicum, Chamaedaphne calyculata, Kalmia polifolia, Andromeda glaucophylla, Vaccinium oxycoccus,* and *Eriophorum spissum* as common associates. The marginal fen includes such species as *Alnus rugosa, Salix discolor, S. serissima, S. gracilis, S. bebbiana, S. pyrifolia, Calamagrostis canadensis, Poa palustris, Carex comosa, C. stricta, C. lasiocarpa, Equisetum silvaticum,* and *Dryopteris cristata,* as well as several elements of the bog flora.

On 24 June 1970, surface organic samples from moderately acid to neutral soils were collected in the Cedar Creek Natural History Area near Minneapolis, the site of the previous study. This repetition was desirable for three reasons: (1) because of the need to determine the pH of the organic soils tested for bacteria, (2) because of a shift in the present work to using a peptone medium for all samples rather than just for pond sediments, and (3) because it seemed worthwhile to test further those sites on the northern side of Cedar Bog Lake which are much less frequently visited by man than the sites on the south side which dominated the previous sampling. At Cedar Creek the old field examined is dominated chiefly by bluegrass (*Poa pratensis*), accompanied by other grasses such as *Sorghastrum nutans, Setaria viridis, S. lutescens, Agropyron repens, Andropogon scoparius, Stipa spartea, Panicum oligosanthes,* and *P. capillare.* Characteristic herbs include *Vicia villosa, Asclepias tuberosa, Lychnis alba, Erigeron canadensis, Chenopodium album,* and *Achillea lanulosa.* Soil samples included varying amounts of surface litter, with the exception of a single sample from a fresh gopher mound. The upland woods contain a mixture of trees such as *Quercus alba, Q. borealis, Pinus strobus, Betula papyrifera, Acer rubrum* and *Ostrya virginiana,* while the lowland fen is dominated by *Thuja* occidentalis close to the upland margin and by *Larix laricina* near the pond. Further floristic data for this transect are given by Gorham and Sanger (1964), who also give data on surface soil pH, water and organic content. Caloric values for soil organic matter are given by Gorham and Sanger (1967).

Enriching and Identifying photosynthetic bacteria

The methods of testing for and identifying Athiorhodaceae were similar to those of the 1970 study, with 2.0 g/liter bactopeptone replacing 2.0 g D-L alanine in the enrichment medium for all tests instead of only those for pond samples. Also, this time sterile bicarbonate was added before rather than after inoculating the enrich-

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ment tubes. All inoculations of enrichment media were made in the laboratory. pH was measured on water squeezed from the soil samples, with dry samples being moistened previously for several hours to a degree roughly similar to that of the wet samples.

Light microscope observation again showed ovoid *Rhodopseudomonas* to be much more common than the spiral-shaped *Rhodospirillum*. Each genus was represented in both fen and bog sites, with no obvious preference.

The colors of the various cultures again ranged widely, from dark red to pink and sometimes orange or yellow. In several tubes inoculated with material from two marginal fen sites, one at Marcell and one at Cedar Creek, a yellow-green color developed very slowly (after 14-26 days, compared to the usual 3-10). Spectrophotometric analysis of these green cultures *in vivo* revealed a strong absorption peak at approximately 1020 nm which is characteristic of bacteriochlorophyll *b*. The far-red peak of the more common bacteriochlorophyll *a* normally appears at 850 to 880 nm *in vivo*. The predominant organism in these green cultures has been isolated and is probably *Rhodopseudomonas viridis* (Pfennig 1967).

Spectrophotometric analysis of pigments extracted from intact cells with 7:2 acetone-methanol revealed considerable diversity of spectra, even more than in the earlier study, and a few examples are shown in Fig. 1. Haskins and Kihara (1967) have claimed that spectra of aqueous cell-free extracts from cultures grown under standardized conditions can be used to differentiate species of Athiorhodaceae.

General confirmation of previous findings

Tests for the presence of Athiorhodaceae are summarized in Table 1. Enrichment with peptone medium confirmed in a general way the results obtained previously with alanine at the Cedar Creek site, with even higher frequencies of occurrence in the pond muds (97 per cent instead of 92 per cent), fen hollows (100 per cent instead of 67 per cent), and fen hummocks (46 per cent instead of 20 per cent). No positives were obtained this time from grassland and woodland sites, whereas low percentages (8 and 14 per cent respectively) were observed previously. On this occasion samples were taken from areas much less travelled by research personnel in the Natural History Area.

Strong soil acidity modifies waterlogging effect

Three acid *Sphagnum* hummocks were sampled in the wetland surrounding Beckman Lake, in the Natural History Area. Two were in a black spruce/tamarack community, and yielded no positives; the third occurred at the margin of this community among sedges and other fen plants, and there one positive tube was recorded.

At Marcell, Athiorhodaceae were similarly scarce in the bog communities. Only one of nine hummocks and one of eleven hollows gave positive results. Humus layers from the jack pine forest gave positive results in only one of nine sites. The marginal fen communities, in contrast, yielded positives in ten out of 12 sites and in 73 per cent of tubes examined.

It is clear once again that the occurrence of Athiorhodaceae is strongly dependent upon the degree of soil waterlogging, but the present study shows that even in suitably wet habitats strongly acid conditions will tend to exclude them. Fig. 2 demonstrates that these photosynthetic bacteria are generally absent in wetland sites below pH 4.7, and present with almost 100 per cent frequency in sites above pH 5.3. Within the pH range 4.7 — 5.3 intermediate levels of frequency are observed. Occasional anomalies are observed, such as a fen hollow (pH 5.2) well out in the bog with no Athiorhodaceae recorded,

TABLE 1. Summary of data on the occurrence of Athiorhodaceae along soil-moisture gradients in acid and circumneutral environments.

Type of Site	pH of Site *	Average soil organic matter (mg./inoc. tube)	Sites positive/ total	Per cent positive sites	Tubes positive/ total	Per cent positive tubes
24 June 1970, Cedar Bog Lake						
Pond mud	6.6-6.8	130	5/5	100	29/30	97
Fen hollow peat	6.3-6.7	520	7/7	100	42/42	100
Fen flat peat	(6.8)	400	1/1	100	6/6	100
Fen hummock peat	(5.5-7.0)	340	2/4	50	11/24	46
Woodland humus layer	(4.2-6.4)	430	0/5	0	0/30	0
Old field surface soils	(6.1-6.6)	250	0/5	0	0/30	0
24 June 1970, Beckman Lake						
Dry *Sphagnum* hummocks	(3.1-3.7)	280	1/3	33	1/9	11
6 November 1969, N of Marcell						
Bog hummock peat	3.6-4.1	300	1/4	25	5/20	25
Bog hollow peat	3.8-5.2	400	1/6	17	1/30	3
Marginal fen (lagg) peat	4.2-5.7	400	4/6	67	18/30	60
Jack pine humus layer	(4.3-4.9)	630	1/4	25	1/20	5
4 June 1970, N of Marcell						
Bog hummock peat	3.8-4.2	240	0/5	0	0/30	0
Bog hollow peat	4.1-4.6	290	0/5	0	0/30	0
Marginal fen (lagg) peat	5.2-5.8	180	6/6	100	30/36	83
Jack pine humus layer	not done	370	0/5	0	0/30	0

* Values in brackets represent dry sites where soils were moistened for analysis.

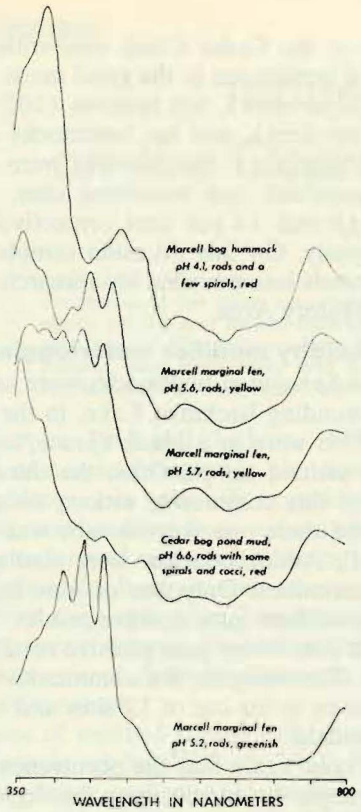

FIGURE 1. Representative absorption spectra of 7:2 acetone-methanol extracts of Athiorhodaceae. Optical density peaks in the region 700-800 nm are from top to bottom, 0.54, 0.50, 0.45, 0.42, 0.32. Amounts of cell material were not standardized

and a *Sphagnum* hummock (pH 4.1) in the bog with 100 per cent presence. Whether the latter case represents accidental animal transport, or an occurrence of an acidophilous species such as the recently described *Rhodopseudomonas acidophila* (Pfennig 1969) remains to be seen.

Observation of the number of days for tubes to show the characteristic colors of Athiorhodaceae suggests that these photosynthetic bacteria are either more active metabolically, or better adapted to the enrichment medium (pH 7.0), when growing in soils above pH 6. With the exception of two unusually slow-growing cultures (one of them *Rhodopseudomonas viridis*), those above pH 6 colored in 5.4 days (standard error $\pm$ 0.5), while those below pH 6 colored in 8.6 days (standard error $\pm$0.5).

Tests with a mixture of cultures from the acid Marcell bog and the circumneutral Cedar Creek fen, in enrichment medium adjusted with hydrochloric acid to a pH range of 2.6-6.8 (the latter being the pH without any acid added), revealed that the Athiorhodaceae grew down to pH 5.3. However, in all but one case the acidity was at least partially neutralized, so that a pH range of 5.6-7.3 was observed in growing cultures by the time the tests concluded. It is perhaps noteworthy that these lower limits span the pH range for declining occurrence shown in Fig. 1, and are close to the range of pH minima (4.8-5.3) given by Pfennig (1969) for growth on succinate by seven strains of his new species *Rhodopseudomonas acidophila.*

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References

DU RIETZ, G. E. 1949. Huvudenheter och huvudgränser i svensk myrvegetation. Summary: Main units and main limits in Swedish mire vegetation. Svensk Bot. Tidskr. *43*: 274-309.

GORHAM, E., and J. SANGER. 1964. Chlorophyll derivatives in woodland, swamp, and pond soils of Cedar Creek Natural History Area, Minnesota, U.S.A., p. 1-12. *In* Y. Miyake and T. Koyama [ed.] Recent researches in the fields of hydrosphere, atmosphere and nuclear geochemistry. Maruzen Co., Tokyo.

_____. 1967. Caloric values of organic matter in woodland, swamp, and lake soils. Ecology *48*: 492-494.

HASKINS, E. F., and T. KIHARA. 1967. The use of spectrophotometry in an ecological investigation of the facultatively anaerobic purple photosynthetic bacteria. Can. J. Microbiol. *13*: 1283-1293.

PFENNIG, N. 1967. Photosynthetic bacteria. Ann. Rev. Microbiol. *21*: 285-324.

_____. 1969. *Rhodopseudomonas acidophila*, sp. n., a new species of the budding purple nonsulfur bacteria. J. Bact. *99*: 597-602.

PRATT, D. C., and E. GORHAM. 1970. Occurrence of Athiorhodaceae in woodland, swamp, and pond soils. Ecology *51*: 346-349.

SJÖRS, H. 1963. Bogs and fens on Attawapiskat River, Northern Ontario. Bull. Nat. Museum Canada, No. 186, Contributions to Botany 1960-61, p. 45-133.

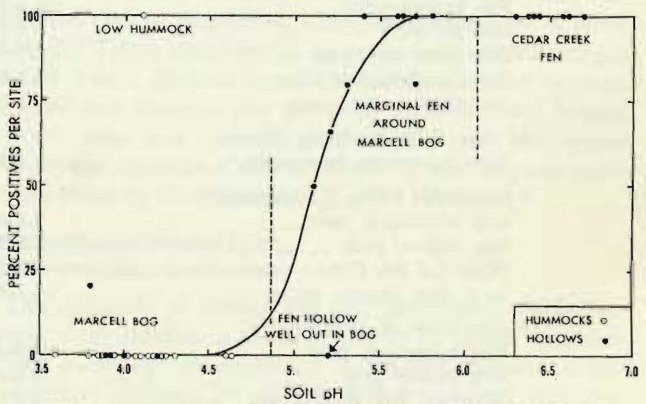

FIGURE 2. The influence of pH on the percentage of positives in wetland sites, excluding dry hummocks (curve fitted by eye).